The Effects of Decontamination Reagents on Collagenous Materials Found in Museums

F. Marte, C. Solazzo, D. W. von Endt, D. Erhardt and C. S. Tumosa

Smithsonian Institution, Washington DC USA

Abstract

Treatments with potent oxidizing agents have been used to neutralize some biological and chemical hazards. Though the decontamination methods are effective, the objects treated are susceptible to damage. The recommended surface chemical treatments include solutions, gels, and foams of oxidizing agents such as peroxides or chlorine bleaching agents. Knowing how these reagents affect various substrates would help to anticipate and minimize any potential damage. This phase of a larger study examines the effects on typical collagenous museum materials of three reagents: hydrogen peroxide, sodium hypochlorite and potassium peroxymonosulfate. Samples of processed animal skins and hide glue were exposed to these reagents. Generally, the treatments yellowed the samples, but specific effects varied with both the substrate and the tested reagent. Amino acid and gas chromatographic analyses have shown that the relative proportions of amino acids are affected by the treatments. At least one amino acid disappears totally after treatment with one of the reagents, presumably through oxidation to other compounds. So far, the observed changes are generally less drastic than might have been expected. Only treatment with peroxide produced changes significantly different from treatment with water alone. However, other materials often associated with the materials tested, such as dyes and inks, are affected, and these reagents should be avoided if safer techniques are available and feasible.

Introduction

In times of emergency it is often necessary to remediate biological or chemical hazards by using potent oxidizing agents to kill microorganisms or neutralize reactive or toxic chemical reagents. It is also necessary that these agents act quickly, usually within thirty minutes; consequently, they must be aggressive in their reaction (Raber and McGuire 2002). This reactivity may extend to the object being treated, which may be irreversibly damaged. The purpose of this study was to test the effects on collagenous materials of three potent reagents, namely hydrogen peroxide, sodium hypochlorite (bleach) and potassium peroxymonosulfate (Oxone®). Treatment in deionized water was also conducted to determine the effects of water alone. For this study, the collagenous materials hide glue, sheepskin parchment, goat vellum and calfskin vellum were tested.

Materials and Methods

Specimens of calfskin vellum, goat vellum and sheepskin parchment were obtained from our reference collection. The specimens are of modern manufacture. The hide glue is from cow skins and was cast as a thin film 8 years previously.

All chemicals were of reagent quality and dissolved in deionized water.

Oxone®, potassium peroxymonosulfate, was donated by the Dupont Corporation, Wilmington DE USA. This is a potent chlorine-free oxidizer.

Five samples were prepared of each specimen: untreated materials were used as controls, a second sample was treated in de-ionized water, a third in a 1% Oxone® solution, a fourth in 3% hydrogen peroxide and the last in sodium hypochlorite diluted 1 to 10 of a 5.8% solution. Sample size was about 4 sq. cm.

Each sample was immersed in a solution for thirty minutes at room temperature.

Amino acid analysis was performed on 1 mg. Each sample was placed into a 12 x 35 mm screw-capped vial, and 200 µl of 6N HCl then added. The vials were flushed three times with dry nitrogen to prevent oxidation and then capped using Teflon® cap liners. The samples were hydrolyzed during 24 h at 100 C. and after hydrolysis were dried under vacuum using a liquid nitrogen trap. The dried samples were then dissolved in 1 ml dilute HCl (2 ml of 12 M HCl in 1 l de-ionized water).

Samples prepared in this way were analyzed directly on a high-performance liquid chromatograph (HPLC) especially designed for ion exchange separation of amino acids. It is similar to the one described by Hare et al. (1985) and in its current modified form described by von Endt (1994). Analysis was performed on a St. John Model 2000 Amino Acid Analyzer with Alcott 708AL Autosampler. Post column derivatization with ortho-phthalaldehyde (OPA) and 2-mercaptoethanol (MCE) as detecting reagents was used. This technique allows for the separation and quantification of most of the amino acids found in proteinaceous materials, however, it does not detect secondary amines such as the two common imino acids proline and hydroxyproline (Benson and Hare, 1975).

For gas chromatographic analysis, 150 µl aliquots from the 1mg/ml solution were taken and dried under vacuum using a liquid nitrogen trap. The dried samples were derivatized with trifluoroacetic anhydride (TFAA) and thionyl chloride. Analysis was conducted on a Hewlett Packard 6890 series gas chromatograph using a nitrogen-phosphorus detector. The column was a Chirasil-Val 25 m x 0.25 mm that enables the separation of D-amino acids from their L-amino acid counterparts.

Results

Treatments were evaluated by comparing the amino acid profile of samples after treatment with the oxidizing reagents with that of the untreated specimen as a control and that of the water treatment as a measure of the effect of water alone, with no oxidizing agent present. The overall results for the four types of substrates tested paralleled each other. The hide glue was solubilized by all of the aqueous reagents tested. For the sake of simplicity only the data for the sheepskin parchment will be presented here.

The effects of a thirty minute immersion of the sheepskin parchment in water are illustrated in Figure 1. The plot shows the before and after concentration in parts per thousand of each of the amino acids measured. There are some slight reductions in the valine, isoleucine and leucine values, possibly as some artifact of the preparation of the parchment, since there is no rise in the concentration of ammonium ion (NH_4^+). Decomposition of amino acids is generally accompanied by an increase in ammonium ion. The decrease in ammonium ion is probably due to washing out of the ion during the thirty minute immersion.

Figure 2 shows the same type of plot of the sheepskin parchment treated with a chlorine based bleach, sodium hypochlorite. The effect on the amino acid distribution is the same as that of water alone. The same type of plot for the Oxone® treatment is shown as Figure 3. This peroxymonosulfate reagent is oxygen-based as opposed to the chlorine-based hypochlorite. Again the change in distribution of the amino acids is similar to that of the previous two treatments. It should be noted that there is also no increase in the decomposition product NH_4^+.

The most dramatic change seen in the tests was with the treatment by hydrogen peroxide, an oxygen based oxidizing agent. Even at 3% concentration this reagent can

alter the amino acid profile of proteins. The effect of peroxide treatment on the sheepskin parchment is shown in the profile displayed in Figure 4. All of the amino acids are reduced and the amount of NH_4^+ decomposition product is greatly enhanced.

The last figure, Figure 5, shows the reduction of the sulfur containing amino acid, methionine, for the sheepskin parchment, goat vellum and calf vellum after each of the treatments. While this amino acid is only present in small amounts, it is interesting that it essentially completely disappears after treatment with Oxone®. Because methionine is oxidized relatively easily, one might expect all of the tested reagents to lower the concentration of methionine. The differences seen may be due more to steric effects than to differences in the potential oxidizing power of the reagents. For example, hypochlorite reduces the concentration of methionine in the insoluble parchment and vellum, but is able to remove all of the methionine only in the hide glue, which dissolves during the treatment.

Discussion

Of the reagents tested, only Oxone® had an overall effect significantly different from treatment with water alone. Oxone® destroyed all of the small amounts of methionine present in the materials tested. While these specific tests have shown no major effects of the oxidizing agents hypochlorite and peroxide on the amino acid profiles of the substrates, submersion in water for thirty minutes with or without the reagents present can cause significant physical changes in the materials. In addition, materials such as inks or dyes often associated with materials such as vellum may be solubilized or oxidized by such treatments. For example, it has been shown in related work in this laboratory that ionizing radiation, another technique used to decontaminate biological agents, causes color changes in at least some of the inks tested (Tumosa et al. 2003). These reagents

are quite strong and the treatments used to apply them potentially quite damaging, and should be used only if no safer or less drastic methods are available or feasible. However, an object may be so severely contaminated or present such an immediate danger that drastic treatment may be necessary. If so, the results of this study should be taken into account in choosing the most appropriate treatment.

Acknowledgments

Part of this work was supported through a grant from the National Center for Preservation Technology and Training, Natchitoches LA, grant # MT-2210-02-NC-07.

References

Benson, J. R., and Hare, P.E., O-Phthalaldehyde: Fluorogenic Detection of Primary Amines in the Picomole Range. Comparison with Fluorescamine and Ninhydrin, Proceedings of the National Academy of Sciences 72:619-622 (1975).

Hare, P. E., St. John, P. A., and Engel, M. H., Ion-Exchange Separation of Amino Acids, in Chemistry and Biochemistry of the Amino Acids, G. C. Barrett, ed., Chapman and Hall, New York, 415-425 (1985).

Raber, E., and McGuire, R., Oxidative Decontamination of Chemical and Biological Warfare Agents using L-Gel, Journal of Hazardous Materials B93:339-352 (2002).

Tumosa, C. S., Erhardt, D. and Solazzo, C., The Effect on Ballpoint Pen and Marker Inks of Chemical and Electron Beam Remediation Techniques for Biological Warfare Agents, MAAFS Newsletter 30(3):5-8 (2003).

von Endt, D. W., Spirit Collections: A Preliminary Analysis of Some Organic Materials Found in the Storage Fluids of Mammals, Collection Forum 10:10-19 (1994).

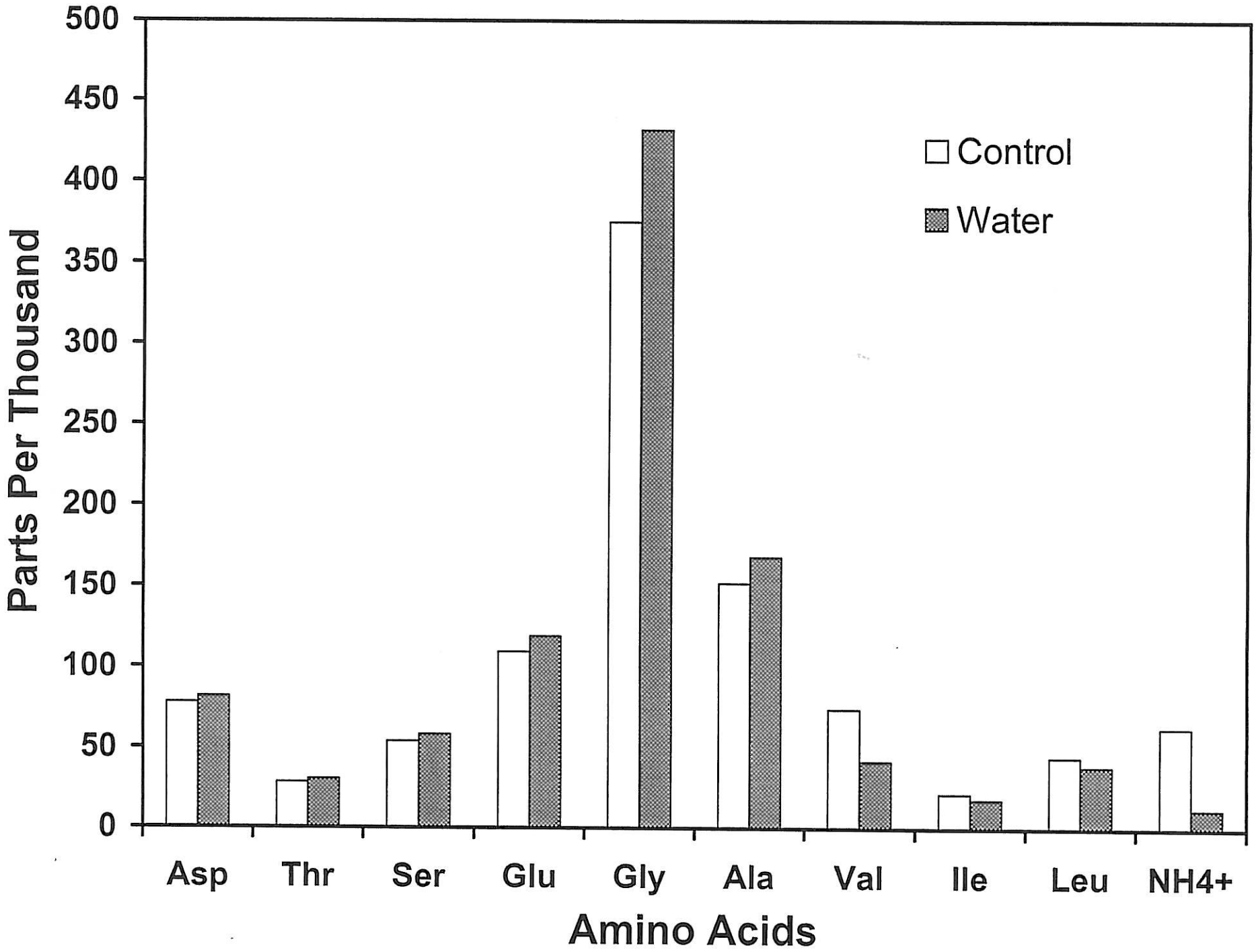
Figure 1 shows the amino acid profile for sheepskin parchment before and after immersion for thirty minutes in water. The amounts are reported as a fraction of the total amount of the listed amino acids detected.

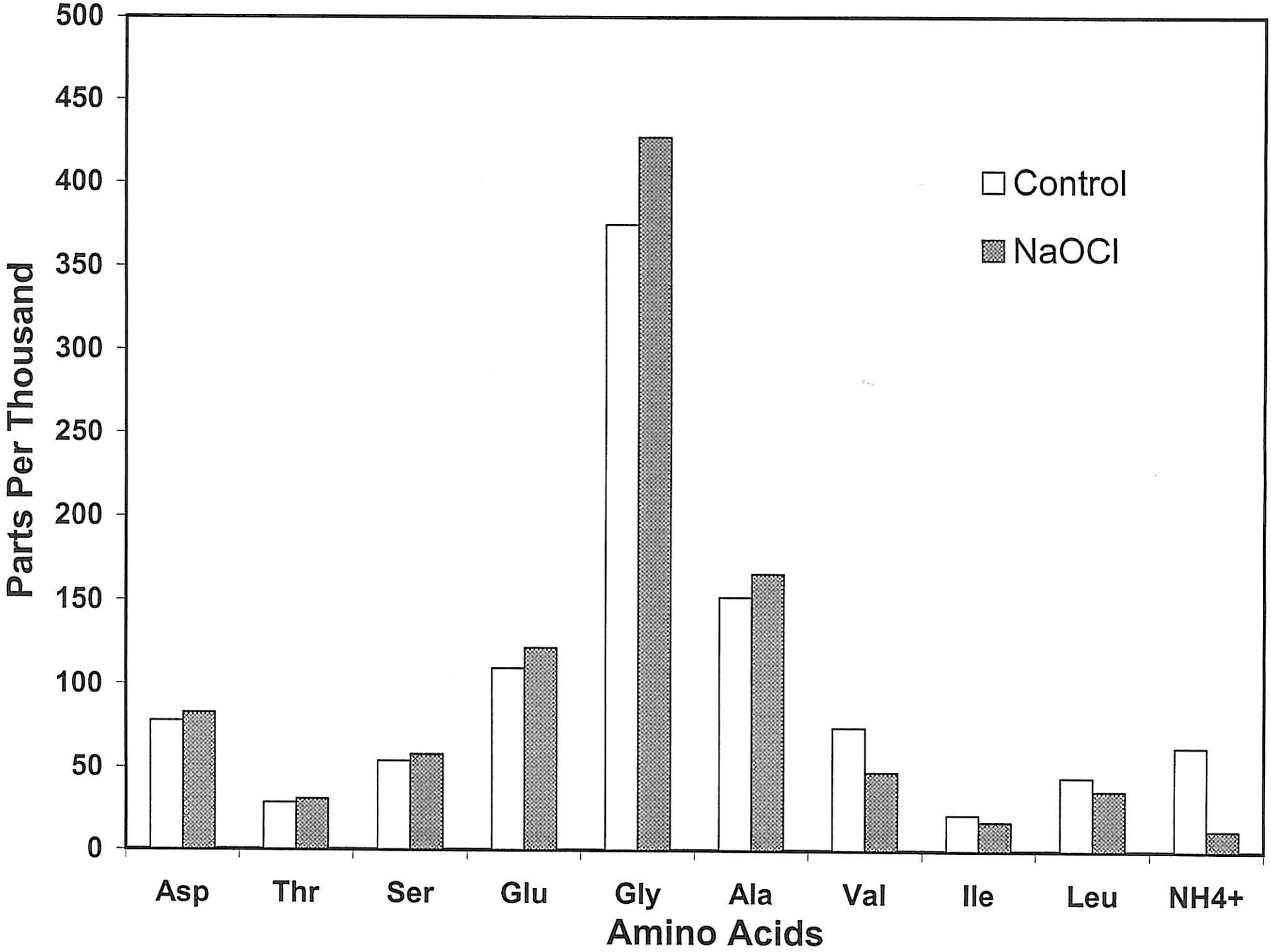
Figure 2 shows the amino acid profile for sheepskin parchment before and after immersion for thirty minutes in sodium hypochlorite bleach diluted 1 to 10. The amounts are reported as a fraction of the total amount of the listed amino acids detected.

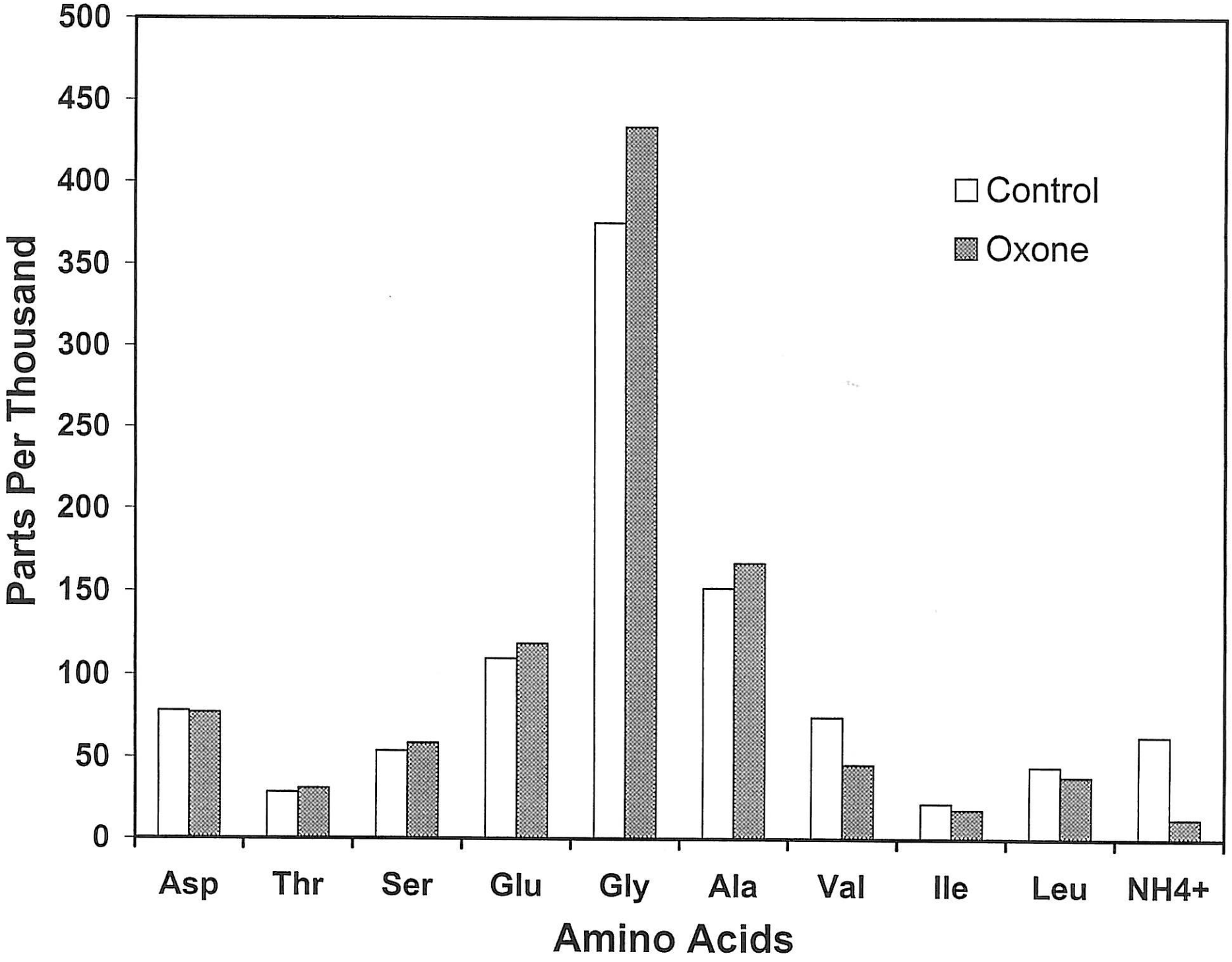
Figure 3 shows the amino acid profile for sheepskin parchment before and after immersion for thirty minutes in a 1% potassium peroxymonosulfate solution. The amounts are reported as a fraction of the total amount of the listed amino acids detected.

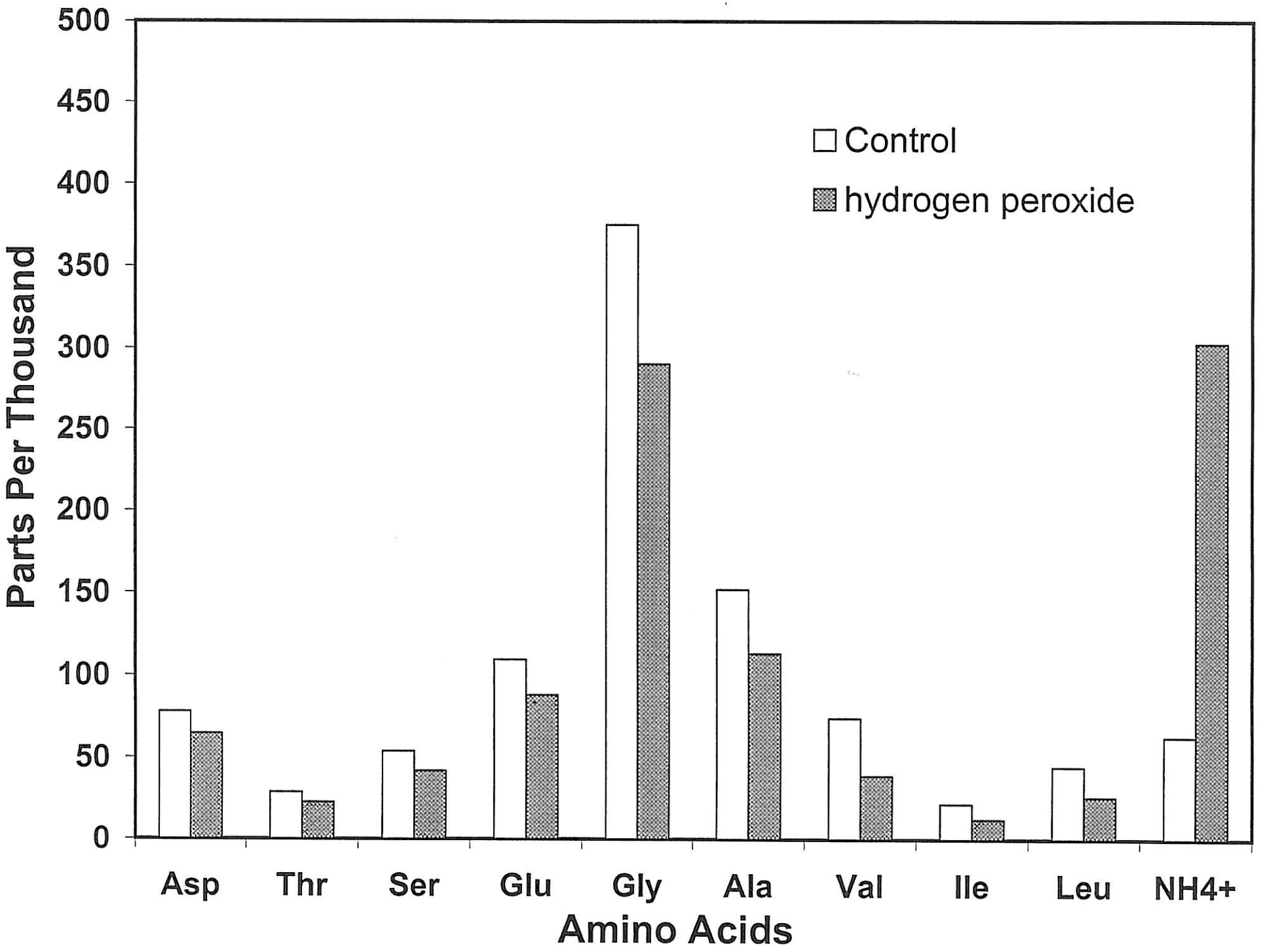
Figure 4 shows the amino acid profile for sheepskin parchment before and after immersion for thirty minutes in a 3% hydrogen peroxide solution. The amounts are reported as a fraction of the total amount of the listed amino acids detected.

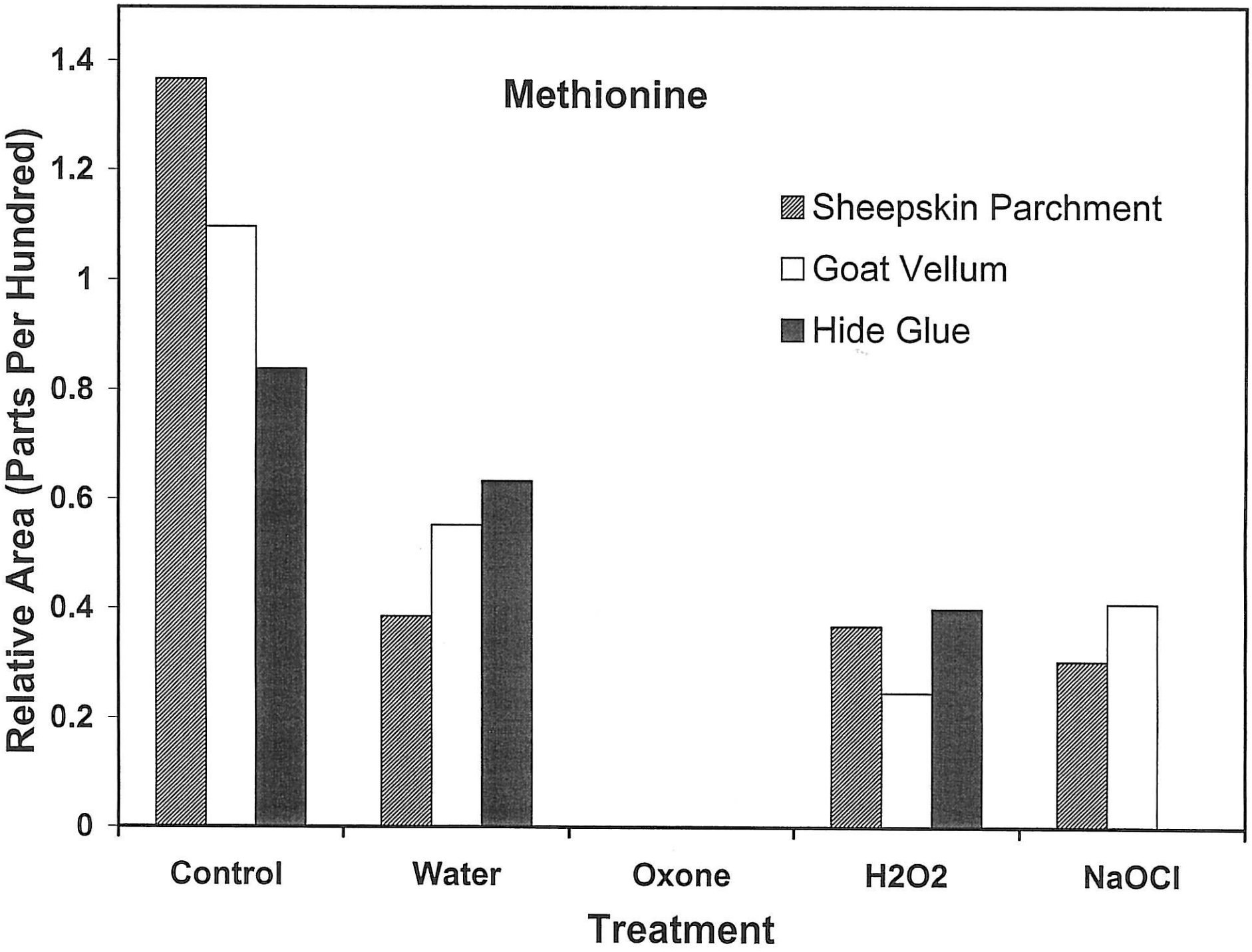
Figure 5 shows the changes in methionine content of sheepskin parchment, goat vellum, and hide glue after thirty minute treatments in water, 1% potassium peroxymonosulfate, 3% hydrogen peroxide, and 1:10 sodium hypochlorite. The amounts are reported as a percent of all material detected. Note the complete loss of methinonine in all peroxymonosulfate treatments.


500
450
400
350
300
250
200
150
100
50
0
Parts Per Thousand
Control
Water
Asp
Thr
Ser
Glu
Gly
Ala
Val
Ile
Leu
NH4+
Amino Acids


500
450
400
350
300
250
200
150
100
50
0
Parts Per Thousand
Control
NaOCl
Asp
Thr
Ser
Glu
Gly
Ala
Val
Ile
Leu
NH4+
Amino Acids


500
450
400
350
300
250
200
150
100
50
0
Parts Per Thousand
Control
Oxone
Asp
Thr
Ser
Glu
Gly
Ala
Val
Ile
Leu
NH4+
Amino Acids


500
450
400
350
300
250
200
150
100
50
0
Parts Per Thousand
Control
hydrogen peroxide
Asp
Thr
Ser
Glu
Gly
Ala
Val
Ile
Leu
NH4+
Amino Acids


Methionine
Sheepskin Parchment
Goat Vellum
Hide Glue
Relative Area (Parts Per Hundred)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
Water
Oxone
H2O2
NaOCl
Treatment